

CLAIMS

We claim:

1. A method of selecting for a plant or portion thereof that comprises a coding region of interest, the method comprising,
 - i) providing a platform plant, or portion thereof comprising a first nucleotide sequence comprising,
 - a first regulatory region in operative association with a first coding region, and an operator sequence, the first coding region encoding a tag protein;
 - ii) introducing a second nucleotide sequence into the platform plant, or portion thereof to produce a dual transgenic plant, the second nucleotide sequence comprising,
 - a second regulatory region, in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region, and;
 - iv) selecting for the dual transgenic plant by identifying plants, or portions thereof deficient in the tag protein, expression of the first coding region, or an identifiable genotype or phenotype of the dual transgenic plant associated therewith.
2. The method of claim 1 wherein the plant or portion thereof comprises plant cells, tissue, or the entire plant.
3. The method of claim 1, wherein the plant, or portion thereof is selected from the group consisting of canola, *Brassica* spp., maize, tobacco, alfalfa, rice, soybean, pea, wheat, barley, sunflower, potato, tomato, and cotton.
4. The method of claim 1, wherein the first coding region is selected from the group consisting of a reporter protein, an enzyme, an antibody and a conditionally lethal coding region.

5. The method of claim 4, wherein the conditionally lethal coding region is selected from the group consisting of indole acetamide hydrolase, methoxinine dehydrogenase, rhizobitoxine synthase, and L-N-acetyl-phosphinothricin deacylase.
6. The method of claim 1, wherein the repressor and the operator sequence are selected from the group consisting of
 - a) Ros repressor and Ros operator sequence;
 - b) Tet repressor and Tet operator sequence;
 - c) Sin3 repressor and Sin 3 operator sequence; and
 - d) UMe6 repressor and UMe6 operator sequence.
7. The method of claim 6 wherein the repressor and the operator sequence are the Ros repressor and Ros operator sequence.
8. The method of claim 6 wherein the repressor and the operator sequence are the Tet repressor and Tet operator sequence.
9. The method of claim 1 wherein the coding region of interest encodes a pharmaceutically active protein.
10. The method of claim 9, wherein the pharmaceutically active protein is selected from the group consisting of growth factors, growth regulators, antibodies, antigens, interleukins, insulin, G-CSF, GM-CSF, hPG-CSF, M-CSF, interferons, blood clotting factors, transcriptional protein or nutraceutical protein.
11. A method of selecting for a transgenic plant or portion thereof comprising a coding region of interest, the method comprising,
 - i) transforming the plant, or portion thereof, with a first nucleotide sequence to produce a transformed plant, the first nucleotide sequence comprising a first regulatory region in operative association with a first coding region, and

an operator sequence, the first coding region encoding a conditionally lethal protein;

ii) screening for the transformed plant;

iii) introducing a second nucleotide sequence into the transformed plant or portion thereof to produce a dual transgenic plant, the second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region, and;

iv) selecting for the dual transgenic plant by exposing the transformed plant and the dual transformed plant to conditions that permit the conditionally lethal coding region to become conditionally lethal, thereby reducing the growth, development or killing the transformed plant.

12. The method of claim 11, wherein the first regulatory region, secondary regulatory region and third regulatory region are constitutively active in the plant cells.
13. The method of claim 11, wherein the first regulatory region and secondary regulatory region are constitutively active and the third regulatory region is developmentally regulated or inducible.
14. A method of selecting for a transgenic plant or portion thereof comprising a coding region of interest, the method comprising,
 - i) introducing a second nucleotide sequence into a transformed plant, or portion thereof that comprises a first nucleotide sequence to produce a dual transgenic plant, the first nucleotide sequence comprising a first regulatory region in operative association with a first coding region, and an operator sequence, the first coding region encoding a tag protein, and wherein the second nucleotide sequence comprises a second regulatory region in operative association with a second coding region, and a third

regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region, and;
ii) selecting for the dual transgenic plant.

15. A method of selecting for a transgenic plant or portion thereof comprising a coding region of interest, the method comprising,
 - i) transforming the plant, or portion thereof, with a first nucleotide sequence to produce a transformed plant, the first nucleotide sequence comprising a first regulatory region in operative association with a first coding region, and an operator sequence, the first coding region encoding a tag protein;
 - ii) screening for the transformed plant;
 - iii) introducing a second nucleotide sequence into the transformed plant or portion thereof to produce a dual transgenic plant, the second nucleotide sequence comprising a second regulatory region in operative association with a second coding region encoding a fusion-protein, the fusion protein comprising a protein of interest fused to a repressor capable of binding to the operator sequence of the first coding region thereby inhibiting expression of the first coding region, and;
 - iv) selecting for the dual transgenic plant.
16. The method of claim 15, wherein the fusion protein additionally comprises at least one of: a) a linker region linking the repressor to the protein of interest and b) an affinity tag.
17. The method of claim 16, wherein the linker region is enzymatically cleavable.
18. The method of claim 15, wherein the fusion protein has a molecular mass below about 100 kDa.

19. The method of claim 15, wherein the fusion protein has a molecular mass below about 65 kDa.
20. A plant cell, tissue, seed or plant comprising,
 - i) a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein, and;
 - ii) a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region.
21. The plant cell, tissue, seed or plant of claim 20, wherein the first coding region is selected from the group consisting of a reporter protein, an enzyme, an antibody and a conditionally lethal coding region.
22. A plant cell, tissue, seed or plant comprising,
 - i) a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein, and;
 - ii) a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, the second coding region encoding a fusion-protein, the fusion-protein comprising a protein of interest fused to a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region.
23. A plant cell, tissue, seed or plant comprising, a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein.

24. A plant cell, tissue, seed or plant comprising, a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to an operator sequence.
25. A construct comprising, a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein.
26. A construct comprising a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to an operator sequence.
27. A pair of constructs comprising,
 - i) a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein, and;
 - ii) a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region.
28. A pair of constructs comprising,
 - i) a first nucleotide sequence comprising a first regulatory region in operative association with a first coding region and an operator sequence, the first coding region encoding a tag protein, and;

ii) a second nucleotide sequence comprising a second regulatory region in operative association with a second coding region, the second coding region encoding a fusion-protein, the fusion-protein comprising a protein of interest fused to a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region.

29. A method of selecting for a plant or portion thereof that comprises a coding region of interest, the method comprising,

i) transforming a plant, or portion thereof with a first nucleotide sequence to produce a transformed plant, the first nucleotide sequence comprising,

a first regulatory region in operative association with a first coding region, and an operator sequence, the first coding region encoding a tag protein;

ii) introducing a second nucleotide sequence into the transformed plant, or portion thereof to produce a dual transgenic plant, the second nucleotide sequence comprising,

a second regulatory region, in operative association with a second coding region, and a third regulatory region in operative association with a third coding region, the second coding region comprising a coding region of interest, the third coding region encoding a repressor capable of binding to the operator sequence thereby inhibiting expression of the first coding region, and;

iv) selecting for the dual transgenic plant by identifying plants, or portions thereof deficient in the tag protein, expression of the first coding region, or an identifiable genotype or phenotype of the dual transgenic plant associated therewith.